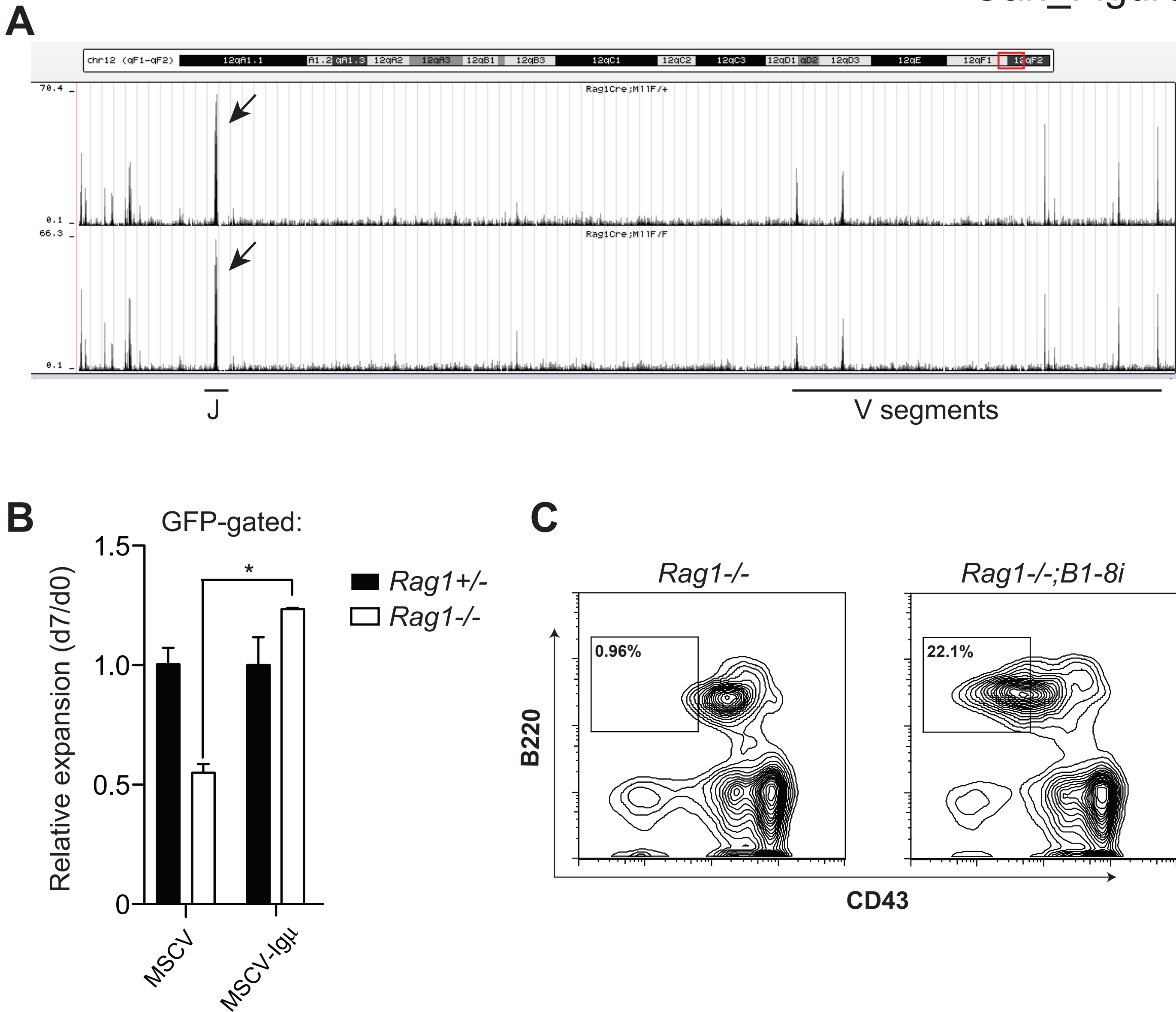

Figure S1. A) Normal levels of H3K4me3 modification at the IgH locus of *Mll1-/-* pro-B cells. Representative H3K4me3 ChIP-seq track from 1 pool of 3 *Mll1*$^{F/F}$ (top) versus 3 *Rag1-cre;Mll1*$^{F/F}$ animals' pro-B cells. Joining (J) and variable (V) regions are labeled below the tracks. **B)** Expression of an Igμ-expressing retrovirus rescues Rag1-deficient pro-B cells. An MSCV-based retrovirus expressing GFP after the internal ribosome entry site was introduced into *Rag1-cre* homozygous (RAG1-deficient) sorted fraction pro-B cells and cells were plated in methylcellulose as described in the Materials and Methods. GFP+CD19+ cells were quantified by flow cytometry after 7 days of culture and are shown as a fraction of the input number of GFP+ cells (relative expansion); *p<0.05. **C)** Representative FACS plots showing the percentage B220+/CD43+ cells in the bone marrow of *Rag1-cre* homozygous mice with or without the B1-8i transgene.

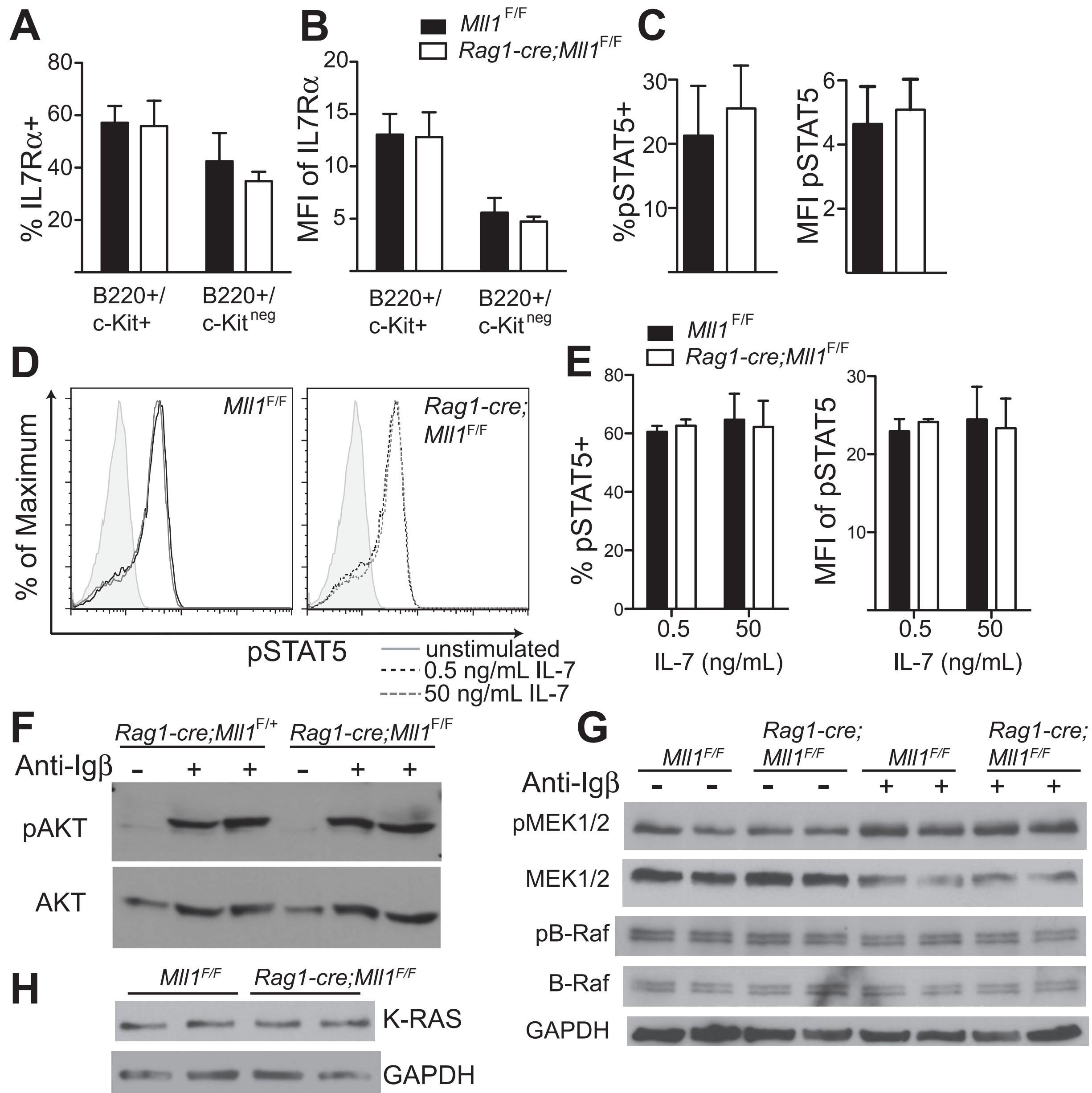

Figure S2. Aspects of IL-7 and pre-BCR signaling that are not affected by *Mll1* deficiency. **A-B)** Expression of the IL7Rα chain (CD127) was determined using 2-3 week old control (*Mll1*$^{F/F}$) or *Rag1-cre;Mll1*$^{F/F}$ animals. Percentage or mean fluorescent intensity (MFI) of IL7Rα is shown as a function of the gated population indicated below each set of bars, n=3 animals per genotype. **C)** STAT5 phosphorylation levels of B220-enriched BM B cells analyzed by flow cytometry after a 20-minute serum starvation followed by a 20-minute, 50 ng/mL IL-7 stimulation showing the percent of pSTAT5-positive cells or MFI of pSTAT5 for 3 mice per genotype. **C)** B220-enriched BM B cells were expanded in vitro for 5 days in 10 ng/mL IL-7, starved without IL-7 overnight, serum-starved for 20 additional minutes, and then stimulated with either 0.5 (solid line) or 50 ng/mL IL-7 (dashed line). Grey histogram represents no added IL-7. **E)** Bar graph quantifies this result using 3 mice per genotype. **F)** CD19+ B cells were isolated from *Rag1-cre;Mll1*$^{F/+}$ and *Rag1-cre;Mll1*$^{F/F}$ animals and expanded for 5 days in 10 ng/mL IL-7. After withdrawal of IL-7 for 4-6 hours, cells were re-stimulated with 30 μg/mL anti-Ig β and the level of AKT and phosphoAKT (S473) was determined by immunoblotting showing two individual animals per genotype. **G-H)** Cells of the indicated genotypes were purified, expanded *in vitro* and stimulated as indicated in (C) except for panel H, which represents unstimulated cells. Western blots were performed with the antibodies indicated to the left of the panels (see Materials and Methods for more detail). Two animals per genotype are shown, representative of two separate experiments.

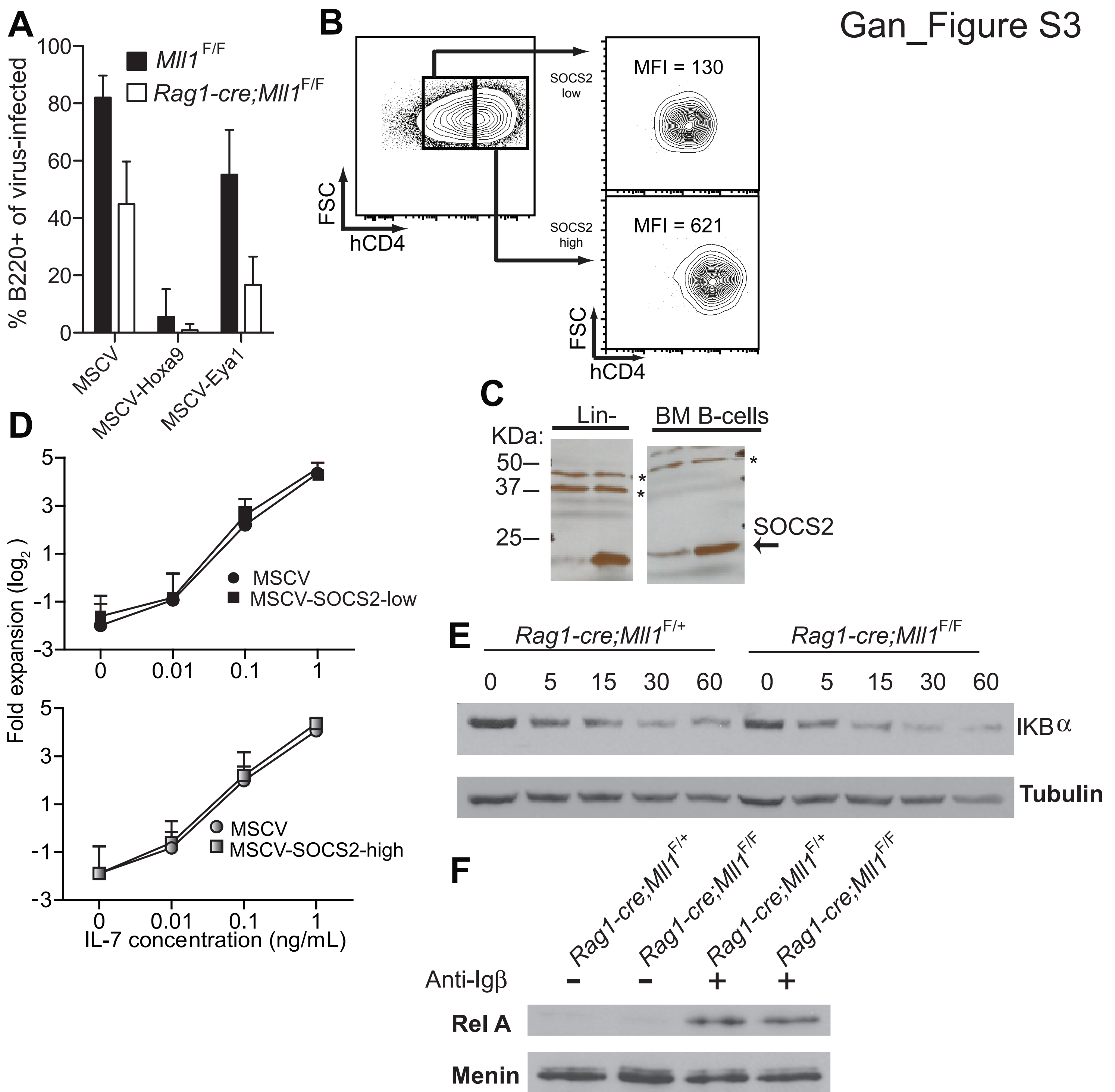

Figure S3. Single gene hypotheses that do not individually account for *Mll1*-deficient B cell phenotypes. **A)** Lineage-depleted BM cells were transduced with a retrovirus expressing human HOXA9 or murine EYA1 as indicated or empty retrovirus (MSCV). Two days later, retrovirus-transduced cells were sorted and plated on methylcellulose and evaluated 7 days later by flow cytometry; the average GFP+/B220+ percentage is shown for triplicate cultures with error bars representing SEM. **B)** Murine SOCS2 was expressed from a bicistronic retroviral vector that co-expresses human CD4 (hCD4). Cells expressing two levels of hCD4 (and hence SOCS2 levels) were isolated.Post-sort analysis is shown to illustrate the distinct levels of hCD4, which corresponded to 1.8 and 2.6 fold overexpression of the Socs2 transcript (data not shown). **C)** Western blot demonstrating overexpression of total SOCS2 from the hCD4 retrovirus in lineage-depleted BM cells (lin-) and CD19-enriched BM B cells. Asterisks show nonspecific bands and arrow indicates SOCS2 band. **D)** Low and high SOCS2 expressing cells were grown in varying concentrations of IL-7 and cell numbers were enumerated at day 4. Fold expansion relative to input is shown for duplicate cultures. **E)** CD19+ B cells were isolated from *Rag1-cre;Mll1*$^{F/+}$ (control) and *Rag1-cre;Mll1*$^{F/F}$ animals and stimulated for 5 days with 10 ng/mL IL-7. After withdrawal of IL-7 for 4-6 hours, cells were re-stimulated with 30 µg/mL anti-Igβ for the minutes indicated above the lane, and the IKBα levels were determined by Western blotting whole cell lysates. **F)** RelA levels were determined using nuclear extracts from cells as in (E), stimulated for 4 hours. This experiment was repeated two additional times with independent animals. Tubulin and Menin are shown as loading controls.